



#25 *[Signature]*
03-24-94

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application : Daniel CAPUT et al.
Serial N° : 07/920,519
Filed : July 28, 1992
For : URATE OXIDASE ACTIVITY PROTEIN,
RECOMBINANT GENE CODING THEREFOR,
EXPRESSION VECTOR, MICROORGANISM
AND TRANSFORMED CELLS
Examiner : David SCHMICKEL
Group Art Unit : 1814

DECLARATION UNDER 37 C.F.R § 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir :

The undersigned, Gérard LOISON, a French citizen, declares as follows :

1. I obtained a PhD in microbiology at the University Pierre et Marie Curie (Paris, France) in 1979 and an Official Doctor's Degree at the University Louis Pasteur (Strasbourg, France) in 1982 (see appendix A). I have been employed by SANOFI, now named ELF SANOFI, since January 1, 1988 as Head of the Laboratory "Expression of Microorganisms" from January 1988 to January 1989 and as Head of the Microbiology Department

since then. I am co-author or co-inventor of the articles and patent applications listed in appendix A.

2. I am a co-inventor of the above-captioned patent application.

3. I have reviewed Reddy et al., Proc. Natl. Acad. Sci. USA 85 : 9081-9085 (1988), a reference cited in the rejection of the claims of the captioned application. The following statements are formed upon my knowledge of this document and my personal knowledge of the field.

4. Reddy et al. relates to the isolation and sequence determination of a cDNA clone for rat peroxisomal urate oxidase. More precisely, a cDNA clone for urate oxidase containing an insert of 1.3 kb is isolated from a λ gt 11 cDNA library prepared from rat liver RNA. The isolation of the mRNAs is performed according to the method described by Chirgwin et al., Biochemistry 18 : 5294-5299 (1979), attached to this declaration as Appendix B. This method involves in particular dissociating animal cell tissues and suspending the dissociated tissues in guanidinium thiocyanate. Thus, in Reddy et al., rat liver cell tissues are dissociated and then suspended in guanidinium thiocyanate, which is sufficient to lyse these cell tissues, which are devoid of walls, and then to free the mRNAs.

5. Such a technique cannot however be applied for the isolation of mRNAs from A.flavus : the dissociation of animal cells, as described by Chirgwin et al., *supra*, does not work with fungal cells because contrary to animal cells, fungal cells do have walls. More particularly, A.flavus has a polysaccharide mycelian wall. The mere dissociation and suspension steps

recited above will therefore not allow the recovery of mycelium from culture of A.flavus. The isolation of mRNAs from A.flavus thus requires a different technique, which has been specifically designed for the present invention and which is described at page 12 of the specification : the mycelium must be frozen in liquid nitrogen, thawed and suspended in lysis buffer, ground with beads to break the mycelium and free the mycelian extract. Due to the great number of polysaccharide particles - corresponding in particular to wall residues - the mRNAs must be then selectively precipitated in the presence of lithium chloride.

6. As explained, the RNA isolation technique described by Chirgwin et al., and used by Reddy et al. to extract mRNAs coding for rat liver urate oxidase, does not make it possible to isolate mRNAs coding for A.flavus urate oxidase.

7. I, hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

03/08/94

Date

Gérard Loison
Gérard Loison.

Gérard LOISON

APPENDIX A

GERARD LOISON

EDUCATION

1979 : PhD degree at the University Pierre et Marie Curie (Paris, France).

Subject : Study of the regulation of yeast (*S. cerevisiae*) URA3 gene.

1982 : Official's Doctor degree at the University Louis Pasteur (Strasbourg, France).

Subject : Expression of *S. cerevisiae* URA1 gene in homospecific and heterospecific environment.

PUBLICATIONS AND PATENT APPLICATIONS

G. LOISON, R. LOSSON & F. LACROUTE (1980)

Constitutive mutants for orotidine-5'-phosphate decarboxylase and dihydroorotic acid dehydrogenase in *Saccharomyces cerevisiae*, *Curr. Genet.*, 2, 39-44

G. LOISON, R. JUND, M. NGUYEN-JUILLERET & F. LACROUTE (1981)

Evidence for transcriptional regulation of dihydroorotic acid dehydrogenase in *Saccharomyces Cerevisiae*, *Curr. Genet.*, 3, 119-123.

F. LACROUTE, ML. BACH, MR. CHEVALLIER, JC. HUBERT, R. LOSSON, D. BOTSTEIN & G. LOISON (1981)

Transcriptional regulation of the yeast pyrimidine genes, *Alfred Benzon Symp.*, 16, 175-181

G. LOISON & R. JUND (1981)

Expression of a cloned *S. Cerevisiae* gene is controlled by a bacterial promoter in *E. coli* and by a yeast promoter in *Saccharomyces Cerevisiae*, *Gene*, 15, 127-137

R. JUND & G. LOISON (1982)

Activation of transcription of a yeast gene in *E. coli* by an IS5 element, *Nature*, 296, 680-682

M. AIGLE, Y. LEMOINE, G. LOISON & JP. LECOQ (1983)

Production of Catechol 2.3. oxygenase by means of yeast, plasmid for the implementation thereof and application (European patent application N° 84902017.7)

R. LATHE, MP. KEINY, Y. LEMOINE, G. LOISON, M. AIGLE & JP. LECOCQ (1983)

Vectors for the expression of a rabbiess antigenic protein in eukaryotic cells and their application to the preparation of a vaccine (European patent application EP-A-0 141 671)

Y. LEMOINE, G. LOISON, P. SONDERMEYER, M. AIGLE & JP. LECOCQ (1984)

Vectors for the expression of interleukin-2 in yeasts, transformed yeasts and method for the preparation of interleukin-2 (European patent application EP-A-0 152 358)

G. LOISON (1984)

Method for preparing a strain, such as a yeast strain, transformed by an expression vector which can be cultured in a complete medium without selection pressure, and strain thus obtained (French patent application FR-A-2 568 891)

G. LOISON, Y. LEMOINE, P. TOLSTOCHEV & JP. LECOCQ
(1985)

Expression and secretion vectors for hirudin by way of transformed yeast (European patent application EP-A-0 200 655)

G. LOISON, M. NGUYEN-JUILLERET, S. ALOUANI & M. MARQUET
(1986)

Plasmid-Transformed URA3FUR1 double mutants of *S. cerevisiae* : an autoselection system applicable to the production of foreign proteins, *Biotechnology*, 4, 433-437

M. MARQUET, S. ALOUANI, ML. HASS, G. LOISON & SW. BROWN
(1987)

Double mutants of *Saccharomyces Cerevisiae* harbour stable plasmids : stable expression of a eukaryotic gene and the influence of host physiology during continuous culture, *Journal of Biotechnology*, 6, 135-145

R. GLOECKLER, G. LOISON & Y. LEMOINE (05/07/87)

Strain of *Saccharomyces* producing α -amylase (European patent application N° 87 401 050.7)

N. LABAT, M. NGUYEN-JUILLERET, G. LOISON & Y. LEMOINE
(06/10/87)

Expression block of an amyloglucosidase in a yeast, transformed yeast and method for preparing an enzyme, and method of fermentation (European patent application N° 87 401 300.6)

M. COURTNEY, E. DEGRYSE, G. LOISON & Y. LEMOINE
(11/30/87)

Hirudin variants, their use and their preparation (European patent application N° 87 402 269.6)

G. LOISON, A. FINDELI, S. BERNARD, M. NGUYEN-JUILLERET,
M. MARQUET, N. RIEHL-BELLON, D. CARVALLO, L. GUERRA-
SANTOS, SW. BROWN, M. COURTNEY, C. ROITSCH & Y. LEMOINE
(1988)

Expression and secretion in *S. cerevisiae* of
biologically active leech hirudin, *Biotechnology*, 6,
72-77

N. RIEHL-BELLON, D. CARVALLO, M. ACKER,
A. VAN DORSSELAER, M. MARQUET, G. LOISON, Y. LEMOINE,
SW. BROWN, M. COURTNEY & C. ROITSCH (1989)

Purification and biochemical characterization of
recombinant hirudin produced by *Saccharomyces*
cerevisiae, *Biochemistry*, 28, 2941-2949.

G. LOISON, A. VIDAL, A. FINDELI, C. ROITSCH,
JM. BALLOUL & Y. LEMOINE (1989)

High level expression of a protective antigen of
schistosomes in *Saccharomyces cerevisiae*, *Yeast*, 5,
497-507

E. JOSEPH-LIAUZUN, P. LEPLATOIS, R. LEGOUX, G. LOISON &
W. ROSKAM (08/24/89)

Peptide signal and DNA sequence coding for said peptide
signal (European patent application N° 89 402 328.2)